

NOVEL TOOLS FOR DIAGNOSIS OF BAKER'S ASTHMA: WHEAT LIPID TRANSFER PROTEIN Tri a 14

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Background: Baker's asthma is among the most important occupational allergic diseases. Wheat lipid transfer protein (LTP) Tri a 14 has been reported as a major allergen associated with wheat allergy. No panel of wheat recombinant allergens for component-resolved diagnosis of baker's asthma is currently performed. We sought to evaluate the potential role of recombinant Tri a 14 as a novel tool for the diagnosis of baker's asthma, and to test the heat and proteolytic resistance of this wheat LTP allergen.

Methods: A cDNA encoding Tri a 14 was isolated and sequenced, the recombinant allergen produced in *Pichia pastoris* and purified by chromatographic methods. Physicochemical and immunological comparison of the natural and recombinant forms of Tri a 14 was carried out by N-terminal amino acid sequencing, matrix-assisted laser desorption/ionization mass spectrometry, circular dichroism analysis, IgE immunodetection, and specific IgE determination and ELISA-inhibition assays using a pool or individual sera from 26 patients with baker's asthma. Thermal denaturation and simulated gastrointestinal digestion of both Tri a 14 forms were checked by spectroscopic and electrophoretic methods, respectively, and biological activity by basophil activation test (BAT).

Results: Natural and recombinant Tri a 14 were similarly folded, as indicated by their nearly identical CD spectra and heat denaturation profiles. A high correlation ($r=0.77$) was found between specific IgE levels to both Tri a 14 forms in individual sera from baker's asthma patients, but a slightly lower IgE-binding potency of rTri a 14 was detected by ELISA-inhibition assays. Natural and recombinant Tri a 14 elicited positive BAT in 2 and 1 out of 3 patients, respectively. Heat denaturation profiles and simulated gastrointestinal digestion assays indicated that Tri a 14 displayed a high heat and digestive proteolytic resistance, comparable to those of peach Pru p 3, the model food allergen of the LTP family.

Conclusions: Recombinant Tri a 14 is a potential tool for baker's asthma diagnosis, based on its physicochemical and immunological similarity with its natural counterpart, and major wheat allergen nTri a 14.